

GENE FLOW FROM FOREIGN PROVENANCES INTO LOCAL PLANT POPULATIONS: FITNESS CONSEQUENCES AND IMPLICATIONS FOR BIODIVERSITY RESTORATION¹

LISÈLE CRÉMIEUX⁴, ARMIN BISCHOFF², HEINZ MÜLLER-SCHÄRER, AND THOMAS STEINGER³

University of Fribourg, Departement of Biology, Unit of Ecology and Evolution, Chemin du musée 10, CH-1700 Fribourg, Switzerland

Long-distance transplantation of seed material as done in restoration programs has raised concerns about the risks associated with the introduction of maladapted genotypes that may hybridize with neighboring native conspecifics and decrease local population fitness (outbreeding depression). We studied the consequences of gene flow from foreign provenances into local populations in the common grassland species *Plantago lanceolata* (Plantaginaceae). Three generations of intraspecific hybrids (F_1 , F_2 , and backcross to the local plants) were produced by controlled crossings between local plants and plants from geographically or environmentally distant populations. Their performance was compared to that of within-population crosses in a field experiment. Early growth in some interpopulation hybrids was significantly reduced, and this decrease in performance was higher in progeny of crosses with the local population from a different habitat than with geographically distant populations. At the end of the growing season, most fitness-related traits of the interpopulation hybrids were close to the average of their parents. Crosses with low-performing foreign parents therefore resulted in reduced fitness of the hybrids compared to the local plants and dilution of local adaptation. We conclude that the introduction of maladapted populations from distant or ecologically distinct environments might, at least temporarily, decrease the fitness of neighboring local plants.

Key words: intraspecific hybridization; large-scale transplantation; local adaptation; outbreeding depression; Plantaginaceae; *Plantago lanceolata*.

Conservation and restoration of biodiversity in agroecosystems has become a priority throughout the world. In restoration schemes of ex-arable land, seed mixtures of several plant species are commonly sown to create species-rich communities. The provenance of seed material used in such restoration programs has raised concerns about how well the introduced genotypes may succeed in novel environments and whether gene flow into native populations may lead to “genetic pollution” (Hufford and Mazer, 2003; McKay et al., 2005). Adaptive genetic differentiation among plant populations resulting from habitat-specific selection (local adaptation) is well documented (Linhart and Grant, 1996; Fenster and Galloway, 2000; Merila and Crnokrak, 2001; Hereford, 2009). Reciprocal transplant studies demonstrating plant local adaptation point at the potential risk of introducing genotypes that are poorly adapted to the local site conditions (Galloway and Fenster, 2000; Montalvo and Ellstrand, 2000; Joshi et al., 2001; Becker et al., 2006a; Bischoff et al., 2006a). Moreover, gene flow from introduced

plants might have negative consequences for the survival of neighboring native populations (Keller et al., 2000).

Intraspecific hybridization between introduced and local populations might lead to reduced fitness in the intraspecific hybrids, referred to as outbreeding depression through two mechanisms: the first one occurs when inherited traits from the foreign population are maladaptive to local site condition (dilution of local adaptation), while the second one is due to the disruption of beneficial epistatic genetic effects following recombination (Lynch, 1991; Hufford and Mazer, 2003 and references therein). This later phenomenon, also referred to as hybrid breakdown, is caused by the disruption of coadapted gene complexes or “intrinsic coadaptation.” Hybrid breakdown is often not evident until the second hybrid generation (F_2) or later, i.e., after intrachromosomal recombination between chromosomes of different origin (Hufford and Mazer, 2003; Edmands, 2007). The environmental component of outbreeding depression or dilution of local adaptation, on the other hand, is more important in the first generation (F_1) of interpopulation hybrids because F_1 hybrids are heterozygous at locally adapted loci (Waser and Price, 1994; Montalvo and Ellstrand, 2001). This negative effect is expected to decrease in subsequent generations, especially if hybrids backcross with the local population (Keller et al., 2000). Moreover, dilution of local adaptation can be partly or wholly compensated by heterosis in F_1 hybrids. Heterosis, or increased vigor relative to the local parental population is caused by either the masking of recessive deleterious alleles or by an overall fitness advantage of heterozygotes (overdominance), as well as by multilocus mechanisms such as positive additive-by-additive interactions (Lynch, 1991). The fitness consequences of hybridization among populations will therefore depend on the relative importance of positive effects of heterosis and negative effects of outbreeding depression and might change across generations (Fenster and Galloway, 2000).

¹ Manuscript received 10 April 2009; revision accepted 18 September 2009.

The authors thank M. Smilauerova, C. Lawson, and S. Mortimer for collecting plants in the Czech Republic and the UK and F. Leuenberger, L. Escher, and R. Zindel for their help in the field. This project was supported by the Swiss Federal Office for Education and Science (project 01.0086 to H.M.-S.) within the Framework V Program of the European Community EVK2-CT-2001-00123 ‘Tlinks’ (Trophic linkages between above- and below-ground organisms as a key to successful restoration of biodiversity on ex-arable land across Europe).

² Current address for Armin Bischoff: National Institute of Horticulture and Landscape Planning (INPH), Department of Biological Sciences, 2 rue André Le Nôtre, F-49045 Angers, France

³ Current address for Thomas Steinger: Agroscope Changins-Wädenswil ACW, Route de Duillier, Case Postale 1012, CH-1260 Nyon 1, Switzerland

⁴ Author for correspondence (e-mail: lisele.cremieux@gmx.fr)

Studies examining the fitness consequences of intraspecific hybridization in most cases have only considered the F_1 generation, and they often found increased vigor of interpopulation hybrids relative to parental fitness (Oostermeijer et al., 1995; Byers, 1998; Sheridan and Karowe, 2000; Luitjen et al., 2002). Most of these studies examined rare species, with small, inbred populations, where outcrossing provides a recovery from genetic drift or inbreeding depression. However, outbreeding depression was also detected in F_1 progeny of crosses among populations when genetic differentiation between the populations was high (Montalvo and Ellstrand, 2001; Pélabon et al., 2005). Studies comparing the performance of F_2 and later generations with parental genotypes are rare (Fenster and Galloway, 2000; Keller et al., 2000), and generally show a pattern of F_1 hybrid heterosis followed by outbreeding depression in later generations. However, effects of genetic introgression may vary considerably depending on life-history traits examined, mating systems and other traits that influence gene flow and levels of among-population differentiation (Montalvo et al., 1997). Outcrossing (self-incompatible) and widely distributed species are expected to be less sensitive to outbreeding depression than species with strong tendency for selfing and small or fragmented populations with low gene flow (Hufford and Mazer, 2003). However, the effect of large-scale interpopulation hybridization in widespread species used in restoration is largely unknown.

We analyzed the effects of gene flow from foreign into local plant populations in the widespread obligate outcrosser *Plantago lanceolata*, for which local adaptation has previously been documented (Bischoff et al., 2006a). We examined the fitness of hybrids between local and two distant (>600 km away) foreign populations. In addition, we included in our crossing scheme a second local population originating from a contrasting habitat. It is generally assumed that negative effects of hybridization increase with increasing geographical distance between populations (Montalvo and Ellstrand, 2001). However, distance is not always a good predictor of adaptive differentiation at small scales (Becker et al., 2006a), and outbreeding depression might be as high in crosses with nearby populations adapted to a different habitat as with very distant populations adapted to the same habitat, depending on the mechanism causing fitness to decrease.

Because hybrid breakdown might not be apparent before the second generation of interpopulation hybrids, we analyzed both F_1 and F_2 offspring. Moreover, to answer our applied objective of investigating how genetic introgression (i.e., the introduction of foreign genes into the population) affects native populations, interpopulation hybrids were also backcrossed to the local population (BC offsprings). Backcross hybrids are likely to be more common than F_2 hybrids in real situations (Edmands, 2007). Even though determining the mechanisms of outbreeding depression was not the primary goal of our study, the comparison of the performance of additional hybrid types beyond the F_1 generation to the parental populations in the field can provide some indications on the relative importance of environmental and physiological factors.

We asked the following specific questions: (1) Is there heterosis (increased fitness) and/or outbreeding depression (reduced fitness) in progeny of crosses between geographically distant populations, as compared to within-population crosses? (2) Is there a higher fitness cost of intraspecific hybridization in the second hybrid generation than in the first generation? (3) Is outbreeding depression more severe in crosses with geographically distant populations than with nearby populations from a different habitat?

MATERIALS AND METHODS

Plant species and populations—*Plantago lanceolata* L. is a short-lived perennial herb, widely distributed in the northern hemisphere, and occurring in a wide range of grassland habitats (Cavers et al., 1980). It is often used in seed mixtures for grassland restoration (Bosshard, 1999). *Plantago lanceolata* can regenerate vegetatively, but it is mainly dispersed by seeds. It is wind-pollinated and protogynous with a gametophytic self-incompatibility system (Ross, 1973). Average distances of gene flow are 0.8–1.5 m for pollen-mediated gene flow, and only 0.04–0.35 m for seed-mediated gene flow (Tonsor, 1985; Bos et al., 1986).

Plants used in experimental crossings were collected from four large grassland populations (population size higher than 500 individuals) that had not been sown in recent decades: a local population (CH1), in close proximity of the experimental site (200 m), two foreign populations (one in the Czech Republic [CZ] and one in England [UK]), and a second local population from a contrasting habitat in Switzerland (CH2) (Table 1). This last population was close to the field site (7 km), as compared to the foreign populations, but from a much drier habitat than the local CH1 population (Table 1). Collections were made in May 2002 by randomly sampling 35 preflowering plants at ~5-m intervals along parallel transects in an area of ~100 m² (~25 m × 40 m).

Experimental crossings—All plants were grown and crossed in the greenhouse. The crossing scheme was designed to simulate a single introgression by foreign pollen. To produce the first generation (F_1) hybrids, we crossed CH1 mother plants with pollen donors from the two foreign origins (CZ and UK), and from the second local habitat (CH2). The inflorescences were covered by pergamin bags before they became receptive. When they were flowering, a spike from the appropriate pollen donor was added to the bag, and the bag was shaken occasionally. The three crossing types were conducted on different inflorescences of the same mother plant and replicated on 24 mother plants, resulting in 72 pollinated inflorescences. A different pollen donor was used for each mother plant. The absence of selfing or contamination was checked with at least one inflorescence per plant: the control inflorescences were covered by pergamin bags but were not hand-pollinated and thus did not produce any seed. The crossing scheme resulted in three types of F_1 hybrids: CH1 × CH2, CH1 × UK, CH1 × CZ, referred to as F_1 -CH2, F_1 -UK and F_1 -CZ hereafter, with 24 half-sib families.

F_1 seeds were germinated in Petri dishes, and 72 seedlings (24 families × 3 origins) were transplanted into pots and grown to adulthood in a greenhouse. The F_1 hybrids were then randomly crossed within each origin to produce F_2 seed: for each F_1 origin, one plant per family was placed in a cage (i.e., 24 plants per cage) covered with two layers of mesh, with the cages separated by at least 2 m to prevent unintended crossings among different hybrid types. Inflorescences were shaken daily. Three types of F_2 hybrids were obtained, referred to as F_2 -CH2, F_2 -UK, and F_2 -CZ (seeds from 21, 17, and 18 plants, respectively). The backcross (BC) generation was produced by pollinating CH1 mother plants with pollen from F_1 progeny of previous crossings (CH1 × F_1). Different CH1 mother plants than the ones used for F_1 seed production were each crossed with all three F_1 types, using three different inflorescences on the same plant, and a different pollen donor for each mother plant. Three types of BC hybrids were obtained: CH1 × (CH1 × UK), CH1 × (CH1 × CZ), CH1 × (CH1 × CH2), referred to as BC-CH2, BC-UK and BC-CZ, each with 11 “families.”

Seeds of the parental generation were produced in parallel under the same greenhouse conditions and with the same set-up as for F_2 seed production (open pollination within origin). P-CH1, P-CH2, P-UK, P-CZ seeds were collected from 18 to 26 mother plants (22 CH1, 19 CH2, 18 CZ, 26 UK).

Germination and field experiment—In May 2004, seeds were germinated in Petri dishes in a growth chamber, with six seeds per mother (total 1476 seeds), grown in trays and transferred to the field 2 weeks later (11–14 June 2004). The field was previously ploughed and kept weed-free throughout the growing season. Twenty-eight blocks containing one plant of each generation and origin (13 plants per block) were set up: 112 parental plants and 84 of each of the three hybrid generations, for a total of 364 plants, were used in the field experiment. In each block, seedlings were planted at distances of 40 cm, large enough to avoid intraspecific competition. Seedlings that did not survive transplanting were replaced by another seedling of the same family (mother plant) within 1 week. Seedling size was estimated at planting as the product of the number of leaves and the length of longest leaf (cumulative leaf length). Plants were harvested aboveground for measurement of total dry mass (vegetative and reproductive parts) at the end of the growing season (November 2004). Inflorescences were counted before harvesting. Three spikes per plant were harvested, and their seeds were counted to obtain an estimate of total seed production.

TABLE 1. Environmental conditions at the collection sites.

Population	Coordinates	Distance to experimental site (km)	Climate (Temperature Jan/Jul; rainfall)	Habitat type (Characteristic species)
CH1	46°52'N, 7°11'E	0.200	Subatlantic (−1.0/17.6°C; 1119 mm/year)	Mesotrophic grassland (<i>Arrhenaterum elatius</i>)
CH2	46°48'N, 7°10'E	7	Subatlantic (−1.0/17.6°C; 1119 mm/year)	Base-rich grassland (<i>Bromus erectus</i>)
CZ	49°0'N, 14°26'E	600	Subcontinental (−1.8/17.7°C; 588 mm/year)	Mesotrophic grassland (<i>Arrhenaterum elatius</i>)
UK	51°40'N, 0°48'W	800	Atlantic (4.3/17.2°C; 651 mm/year)	Chalk grassland (<i>Arrhenaterum elatius</i>)

Data analysis—The effects of generation and origin on seedling size, aboveground vegetative biomass and seed production were examined using mixed-effect ANOVA models using the program JMP 5.0.1 (SAS, Cary, North Carolina, USA). Models contained fixed effects due to generation (P , F_1 , F_2 , BC) and origin (CH1, CH2, UK, CZ) nested in generation, random effect due to block, and the following covariates: seed mass for the dependent variable size-at-planting, size at planting for the dependent variables biomass and seed production. Vegetative biomass and seed production were square-root transformed to meet the assumptions of normality and homoscedasticity. Because the covariate size-at-planting was highly significant, only plants that were not replaced just after planting were included in the analysis; however, removing the covariate from the model and including all plants did not change the results. Dead plants ($N = 2$) were excluded from the analysis.

We calculated a series of a priori contrasts within significant origin effects to test for deviation from additive gene action. The performance of the F_1 and F_2 hybrids was compared to the mean performance (Pf) of their parents (midparent value: $1/2 \cdot Pf_{CH1} + 1/2 \cdot Pf_{Foreign}$). Expected values for backcrosses were calculated as $3/4 \cdot Pf_{CH1} + 1/4 \cdot Pf_{Foreign}$ (Etterson et al., 2007). For each hybrid generation, we calculated an overall contrast comparing the hybrids with their parents for all three origins (1 contrast with $df = 3$ for each generation). If this overall contrast was significant, we tested the hybrid vs. parental generation separately for each origin (3 contrasts with $df = 1$). The weightings for the contrasts assume additive gene action, i.e., they test whether heterotic (dominance) effects outweigh the breakdown of positive allele combinations due to hybridization. Positive deviation from additive gene action would indicate heterotic effects (hybrid vigor), while negative deviation indicates hybrid breakdown.

Even though we cannot fully test for local adaptation in this experiment, we checked whether the local (CH1) population performed better than the two foreign (CZ, UK) and the second local (CH2) populations using a priori contrasts specified within the origin effect in a separate model including only the parental plants. Random effects due to block and the same covariates as described were included in the models.

RESULTS

Local vs. foreign plants—Differences between local (CH1) and foreign parental plants (CZ, UK) were small at the start of the experiment (contrast for seedling size: $F_{1,77} = 1.96$, $P = 0.166$), but local plants produced significantly more vegetative biomass ($F_{1,74} = 4.09$, $P = 0.047$) and more seeds ($F_{1,74} = 9.81$, $P = 0.002$) compared with foreign plants at the end of the growing season. Vegetative biomass of the CZ and UK plants was, respectively, 36% and 25% lower than biomass of the CH1 plants, and CZ plants produced 23% fewer seeds than the local plants. No differences were found between the local (CH1) and the contrasting-habitat (CH2) populations in any of the measured traits (seedling size: $F_{1,77} = 2.33$, $P = 0.131$; biomass: $F_{1,74} = 1.89$, $P = 0.173$; seed production: $F_{1,74} = 0.29$, $P = 0.589$).

Hybrid vigor in F_1 generation?—Generation had significant effects on seedling size measured at the time of planting in the field as well as on seed production. Origin (nested within generation) had significant effects on all three measured performance traits (Table 2). We found no evidence for hybrid vigor in between-population hybrids for any of the measured performance traits. The overall contrasts specified within the origin effect showed significantly smaller seedling size of the F_1 hybrids compared to the average of their parents (Table 2).

However, when testing separately for each origin, the differences were only significant in the CH2 hybrids (Fig. 1). For vegetative biomass and seed production, the contrasts testing for deviation from additive gene action were all nonsignificant (Table 2). Performance of the interpopulation hybrids was close to the average of their parents, resulting in reduced fitness of the hybrids relative to the home parent when the foreign parent showed low fitness. For example, crosses with the poorly performing CZ population resulted in 28% reduction of vegetative biomass and in a 17% lower seed production compared to the CH1 population (Figs. 2, 3).

Outbreeding depression in F_2 and BC generations?—Seedling size of the F_2 hybrids was significantly inferior to the midparent value, overall and in the CH2 and UK origins (Table 2, Fig. 1). However, these effects did not persist because contrasts testing for deviation from additive gene action in vegetative biomass of adult plants were nonsignificant (Fig. 2). The performance of the BC hybrids also did not differ from expectations, assuming additive effects for seedling size and final vegetative biomass. For seed production, effects of interpopulation crossing varied among origins. Indeed, performance of later generation hybrids (F_2 and BC) was significantly higher than expected assuming additive genetic effects in crosses with the CZ population, while there were no epistatic effects in CH2 and UK hybrids (Fig. 3).

DISCUSSION

We investigated the effects on offspring performance of hybridization between a local Swiss population of *P. lanceolata* and geographically or environmentally distant populations. In a

TABLE 2. Effect of generation and origin (nested in generation) on plant performance (F -values and significance).

Effect	df	Seedling size $N = 355$	Biomass $N = 342$	Seed production $N = 341$
Generation	3	7.45***	1.54	6.23**
Origin[Generation]	9	2.65**	2.71**	4.62***
F_1 vs. MP	3	3.84*	1.00	1.20
F_2 vs. MP	3	6.77**	1.54	4.14**
BC vs. $3/4 \cdot Pf_{CH1} + 1/4 \cdot Pf_{Foreign}$	3	0.24	0.06	2.53(*)
Covariate ^a	1	70.58***	8.67**	41.96***

Notes: Linear contrasts were specified within significant origin effects to test for deviation from additive gene action, that is represented by the midparent value (MP). Vegetative biomass and seed production were square-root transformed before analysis. Block effects were included in the model (not shown). MP: midparent value, F_1 : first hybrid generation, F_2 : second hybrid generation, BC: backcross generation, Pf_{CH1} : performance of the recurrent parent for backcrossing (CH1), $Pf_{Foreign}$: performance of the foreign parental origin in the backcross. Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, (*) $P = 0.06$.

^a Significant covariate included in the model: seed mass for seedling size, seedling size for biomass and seed production.

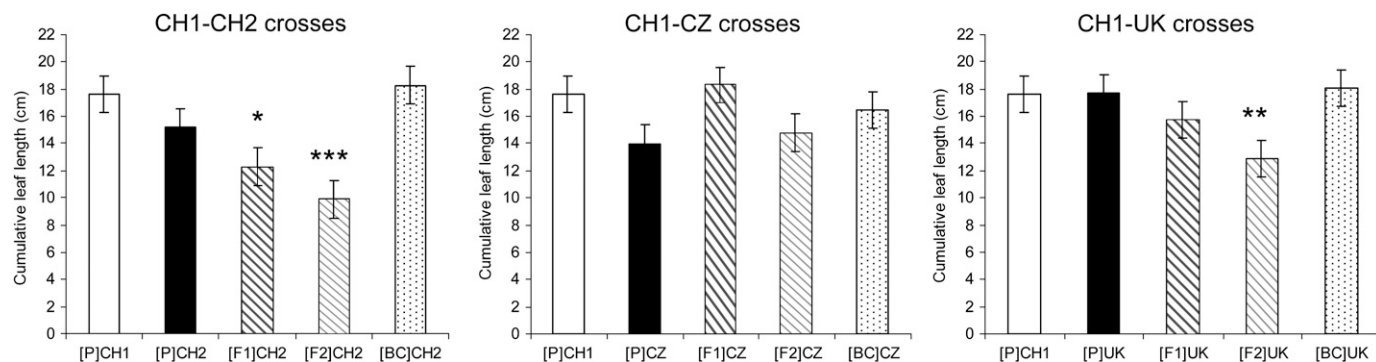


Fig. 1. Seedling size (cumulative leaf length) of the parental and hybrid generations of *Plantago lanceolata* as measured by the number of leaves times the length of the longest leaf after 2 weeks of growth in a climate chamber (least squared means \pm SE). CH1 is the local population, CZ and UK are geographically distant populations, CH2 is a nearby population from a different habitat. The degree of shading represents the proportion of foreign genes. Deviation from additive gene action in the interpopulation hybrids was tested using a priori contrasts within significant origin[generation] effects: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

previous study using reciprocal transplant experiments, we had demonstrated local adaptation across a broad geographical range, as well as strong population differentiation among diverse habitats in this widespread outcrossing species (Bischoff et al., 2006a). The current study confirmed superior performance of the home population at our experimental site. Interpopulation hybridization had negative effects on seedling growth in crosses with two of the three populations. These effects were stronger in the F_2 generation and in crosses with the local population from a different habitat. However, vegetative biomass and seed production at the end of the growing season did generally not differ from expectations assuming additive gene effects.

No evidence for hybrid breakdown in the field—Outbreeding depression, or lower performance of interpopulation hybrids, can be caused by breakdown of coadapted gene complexes and/or dilution of local adaptation (Lynch, 1991; Huford and Mazer, 2003). The latter component of outbreeding depression appears when phenotypic traits are not well adapted to the local environment, while the physiological component, also referred to as hybrid breakdown, is due to intrachromosomal recombination between chromosomes of different origins and would also be apparent in a neutral environment.

Distinguishing between the two components of outbreeding depression would require reciprocal transplant experiments and reciprocal crossings (Fenster and Galloway, 2000; Montalvo and Ellstrand, 2001), which was beyond the scope of this experiment. However, the performance of foreign parents relative to local plants provides an indication of their degree of maladaptation to the local conditions at the experimental site. Moreover, the contrasts tested for deviation from additive gene effects, with the effect of foreign, less-adapted genes included in the expected value (the performance of the hybrid is compared to the average of its parents). Significant differences from expectations would indicate a role of positive heterotic (dominance) effects and/or the breakdown of positive allele combinations (negative epistatic effects) in determining hybrid fitness.

In this experiment, negative effects of interpopulation hybridization were only found in seedling growth (size at planting) and were stronger in the F_2 generation. Because plants were germinated and grown in a growth chamber for 2 weeks, lower performance of the CH2 and UK interpopulation hybrids suggests negative intra- and/or interlocus interactions (epistasis) resulting from combining genes from different populations rather than dilution of local adaptation. However, these effects decreased during the growing season in the field, and the final

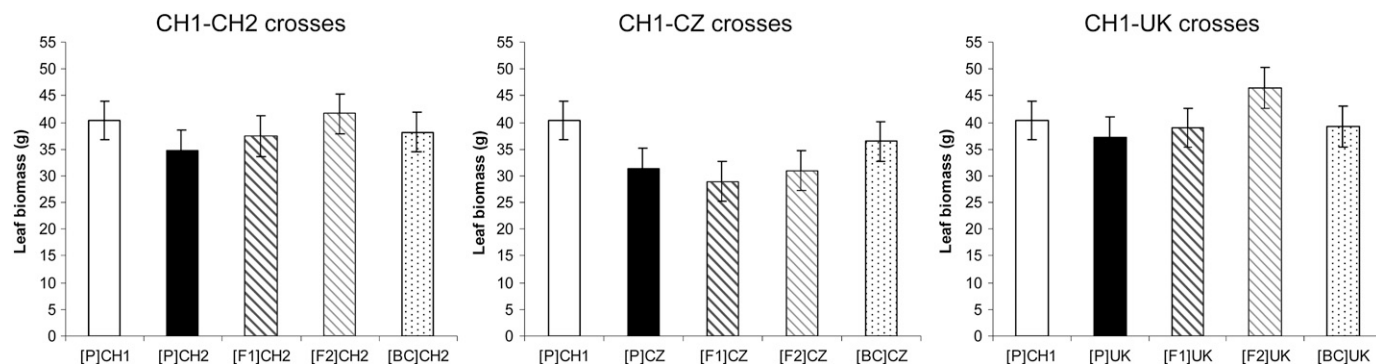


Fig. 2. Vegetative biomass of the parental and hybrid generations of *Plantago lanceolata* for the three crossing origins after one growing season in the field (least squared means \pm SE). CH1 is the local population, CZ and UK are geographically distant populations, CH2 is a nearby population from a different habitat. The degree of shading represents the proportion of foreign genes. Deviation from additive gene action in the interpopulation hybrids was tested using a priori contrasts within significant origin[generation] effects.

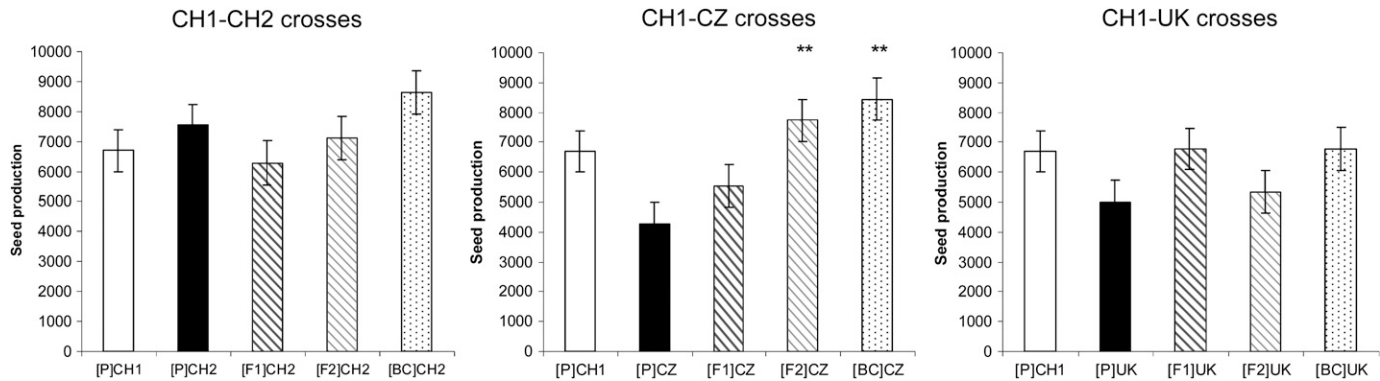


Fig. 3. Seed production of the parental and hybrid generations of *Plantago lanceolata* for the three crossing origins after one growing season in the field (least squared means \pm SE). CH1 is the local population, CZ and UK are geographically distant populations, CH2 is a nearby population from a different habitat. The degree of shading represents the proportion of foreign genes. Deviation from additive gene action in the interpopulation hybrids was tested using a priori contrasts within significant origin[generation] effects: ** $P < 0.01$.

biomass and seed production of the hybrids did not differ from expectations assuming additive gene effects. This lack of negative epistatic effects in the first two generations of interpopulation hybrids grown in the field indicates greater importance of the ecological component as compared to the physiological component in determining hybrid fitness. The nonsignificant deviation from additive effects could also be explained by heterosis due to dominance or overdominance, which could compensate for the negative consequences of interrupting epistatic interactions (Johansen-Morris and Latta, 2006). However, this scenario is not likely to be the case here because we found no evidence for hybrid vigor in the F_1 hybrids. This lack of heterosis indicates that populations do not suffer from inbreeding depression (e.g., due to the outcrossing breeding system and large population sizes). It is also possible that fitness costs of hybridization would become apparent only in the F_3 or later generations, a pattern to be expected when there is strong linkage of coadapted loci. Fenster and Galloway (2000) observed fitness costs of hybridization between populations of the annual outcrossing plant *Chamaecrista fasciculata* in the F_3 generation, while effects in earlier generations were additive or even heterotic. Even though we monitored the plants for a complete growing season, it may still be possible that our measure of fitness did not take into account the critical stages for outbreeding depression. In our study, plants germinated under favorable conditions in a growth chamber, resulting in high germination rates and no deviation from additive effects in the contrasts testing for hybridization effects in germination (not shown). Although population differentiation in the field was found to be similar to that in growth chambers (Bischoff et al., 2006b), a different response due to hybrid type-by-environment interactions cannot be excluded if seeds had been directly sown in the field. Moreover, the plants of the current study were grown under noncompetitive conditions, and the observed differences in early growth might have increased under competition. Bischoff et al. (2006a) showed that adaptive differentiation in *Plantago lanceolata* was stronger with competition in a plant community than without competition. However, Willi et al. (2007) found no effect of competitive stress on the pattern of heterosis and (lack of) outbreeding depression. If the fitness effects of breaking down coadapted gene complexes were strong in these populations, then they should be apparent in fitness components such as vegetative biomass and seed production.

Population differentiation and dilution of local adaptation—The performance of the interpopulation hybrids was close to the average of their respective parents. Local parents had the highest performance, and dilution of local adaptation, with lower vegetative growth and reproduction of the F_1 hybrids compared to the local genotypes was found in crosses with a poorly performing foreign population. With additive gene action, a blurring of genetic differences associated with local adaptation is indeed expected after hybridization: if introduced and local genotypes are adapted to different environments and have become fixed for different alleles, the F_1 hybrids will be heterozygous at locally adapted loci, resulting in a 50% “dilution” of the differently adapted parental genomes and a lower fitness of the hybrids compared to the local parent grown in its native environment (Hufford and Mazer, 2003). Such dilution of local adaptation was found in F_1 hybrids of *Delphinium nelsonii* (Waser and Price, 1994) and *Lotus scoparius* (Montalvo and Ellstrand, 2001).

In this study, differences between local and foreign populations of *P. lanceolata*, as well as with the second local population were smaller than previously found (Bischoff et al., 2006a), probably because the plants were grown in the absence of competition. Superiority of the local provenance was found to be larger in competition with the local plant community (Bischoff et al., 2006a). Such high levels of maladaptation in foreign populations would lead to the formation of low-performing interpopulation hybrids relative to the local plants and significant decrease of native population fitness.

In the absence of further genetic disruption, effects of dilution of local adaptation are likely to decrease, especially in backcrosses with the local population. Indeed, while there was a trend for decreased or average fitness of the F_1 and/or F_2 interpopulation hybrids, performance of the BC generation was always as high as the local population. The proportion of foreign, maladapted genes decreases rapidly in backcrosses with the local population, while fitness recovery in crosses among the F_1 progeny depends on the time required to restore homozygosity at loci adapted to the local environment (Hufford and Mazer, 2003).

Geographic distance vs. habitat differences—We studied the effects of intraspecific hybridization in crosses between populations separated by large geographic distances but

occurring in similar habitats. The effects were compared to crosses with a nearby population from a contrasting (drier) habitat. Negative consequences of hybridization are assumed to increase with geographic distance between populations because there is often a strong correlation between genetic and geographic differences on the large geographical scale (Fenster and Dudash, 1994; Becker et al., 2006a). However, genetic divergence among populations is not necessarily linked with geographic distance (Montalvo and Ellstrand, 2001; Petit et al., 2001), and adaptive differentiation should also reflect local environmental heterogeneity.

In this experiment, significant deviations from additive gene action, in the direction of decreased fitness, were only found in early growth of the F_1 and F_2 progeny of crosses between different habitats at the local scale, and in the F_2 progeny of one long-distance cross type. This result suggests that distance between populations is not a good predictor of the magnitude of hybrid breakdown. Keller et al. (2000) and Becker et al. (2006b) also found that the extent of outbreeding depression did not increase with interpopulation distance, while Galloway and Etterson (2005) found that F_1 hybrid performance decreased with increasing distance between populations. Becker et al. (2006a) concluded that distance is a good indicator of the extent of adaptive differentiation at large (>200 km) but not at small scales.

Even though performance of interpopulation hybrids in the field did not show strong outbreeding depression, potential for dilution of local adaptation in crosses with local population from a different habitat is as high as in crosses with foreign populations, as indicated by results from Bischoff et al. (2006a) with the same populations. Many species show strong population differentiation and local adaptation at small scale (Waser and Price, 1985; Galen et al., 1991; Van Tienderen, 1992; Kindell et al., 1996; Lenssen et al., 2004). Therefore, it might be more important to maximize environmental similarities of source populations relative to transplant site than to minimize geographic distance, both for the success of the restored area (Raabova et al., 2007) and for avoiding a decrease in the mean population fitness of neighboring local populations.

Conclusions—Large-scale translocation of plants species to restore diverse ecosystem has raised concerns about the risks associated with the introduction of maladapted genotypes that may hybridize with locally adapted conspecifics and lead to outbreeding depression (Hufford and Mazer, 2003). Here we show that additive gene action governs performance of the intraspecific hybrids in a widespread, common plant. This result suggests that the introduction of maladapted populations might, at least temporarily, decrease the fitness of neighboring local plants. In the absence of further genetic disruptions, the effects of dilution of local adaptation are likely to decrease over time. However, the long-term effects of large-scale introductions on local population mean fitness are hard to predict. Indeed, the degree to which natural selection effectively eliminates poorly adapted genotypes resulting from intraspecific hybridization is unknown (Hufford and Mazer, 2003). Moreover, repeated introductions over several years, as in the case of plant species sown in grasslands restoration, will counteract local adaptation and continue to negatively impact the fitness of local populations (Keller et al., 2000). The choice of an appropriate provenance is therefore of prime importance if negative consequences for local populations are to be avoided. In addition to geographic distance, environmental similarities between restoration and donor site should be considered when choosing source

material. More research on the patterns of outbreeding depression that are relevant to restoration and their consequences for population persistence in widespread and rare plant species is needed to determinate the generality of these findings.

LITERATURE CITED

- BECKER, U., G. COLLING, P. DOSTAL, A. JAKOBSSON, AND D. MATTHIES. 2006a. Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. *Oecologia* 150: 506–518.
- BECKER, U., T. REINHOLD, AND D. MATTHIES. 2006b. Effects of pollination distance on reproduction and offspring performance in *Hypochoeris radicata*: Experiments with plants from three European regions. *Biological Conservation* 132: 109–118.
- BISCHOFF, A., L. CRÉMIEUX, M. SMILAUEROVA, C. S. LAWSON, S. R. MORTIMER, J. DOLEZAL, V. LANTA, ET AL. 2006a. Detecting local adaptation in widespread grassland species—The importance of scale and local plant community. *Journal of Ecology* 94: 1130–1142.
- BISCHOFF, A., B. VONLANTHEN, T. STEINGER, AND H. MÜLLER-SCHÄRER. 2006b. Seed provenance matters—Effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology* 7: 347–359.
- BOS, M., H. HARMENS, AND K. VRIELING. 1986. Gene flow in *Plantago*. I. Gene flow and neighborhood size in *P. lanceolata*. *Heredity* 56: 43–54.
- BOSSHARD, A. 1999. Restoration of species rich meadows on former intensively used farmlands. *Dissertationes Botanicae* 303: 1–194.
- BYERS, D. L. 1998. Effect of cross-proximity on progeny fitness in a rare and a common species of *Eupatorium* (Asteraceae). *American Journal of Botany* 85: 644–653.
- CAVERS, P. B., I. J. BASSETT, AND C. W. CROMPTON. 1980. The biology of Canadian weeds. 47. *Plantago lanceolata*. L. *Canadian Journal of Plant Science* 60: 1269–1282.
- EDMANDS, S. 2007. Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* 16: 463–475.
- ETTERSON, J. R., S. R. KELLER, AND L. F. GALLOWAY. 2007. Epistatic and cytonuclear interactions govern outbreeding depression in the autotetraploid *Campanulastrum americanum*. *Evolution* 61: 2671–2683.
- FENSTER, C. B., AND M. R. DUDASH. 1994. Genetic considerations for plant population restoration and conservation. In M. L. Bowles and C. J. Whelan [eds.], *Restoration of endangered species: Conceptual issues, planning and implementation*, 34–62. Cambridge University Press, Cambridge, UK.
- FENSTER, C. B., AND L. F. GALLOWAY. 2000. Population differentiation in an annual legume: Genetic architecture. *Evolution* 54: 1157–1172.
- GALLEN, C., J. S. SHORE, AND H. DEYOE. 1991. Ecotypic divergence in alpine *Polemonium viscosum*: Genetic structure, quantitative variation, and local adaptation. *Evolution* 45: 1218–1228.
- GALLOWAY, L. F., AND J. R. ETTERSON. 2005. Population differentiation and hybrid success in *Campanula americana*: Geography and genome size. *Journal of Evolutionary Biology* 18: 81–89.
- GALLOWAY, L. F., AND C. B. FENSTER. 2000. Population differentiation in an annual legume: Local adaptation. *Evolution* 54: 1173–1181.
- HEREFORD, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist* 173: 579–588.
- HUFFORD, K. M., AND S. J. MAZER. 2003. Plant ecotypes: Genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution* 18: 147–155.
- JOHANSEN-MORRIS, A. D., AND R. G. LATTI. 2006. Fitness consequences of hybridization between ecotypes of *Avena barbata*: Hybrid breakdown, hybrid vigor, and transgressive segregation. *Evolution* 60: 1585–1595.
- JOSHI, J., B. SCHMID, M. C. CALDEIRA, P. G. DIMITRAKOPOULOS, J. GOOD, R. HARRIS, A. HECTOR, ET AL. 2001. Local adaptation enhances performance of common plant species. *Ecology Letters* 4: 536–544.
- KELLER, M., J. KOLLMANN, AND P. J. EDWARDS. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology* 37: 647–659.

- KINDELL, C. E., A. A. WINN, AND T. E. MILLER. 1996. The effects of surrounding vegetation and transplant age on the detection of local adaptation in the perennial grass *Aristida stricta*. *Journal of Ecology* 84: 745–754.
- LENSSEN, J. P. M., M. VAN KLEUNEN, M. FISCHER, AND H. DE KROON. 2004. Local adaptation of the clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. *Journal of Ecology* 92: 696–706.
- LINHART, Y. B., AND M. C. GRANT. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27: 237–277.
- LIJUTEN, S. H., M. KÉRY, J. G. B. OOSTERMEIJER, AND H. C. M. DEN NIJS. 2002. Demographic consequences of inbreeding and outbreeding in *Arnica montana*: A field experiment. *Journal of Ecology* 90: 593–603.
- LYNCH, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution; International Journal of Organic Evolution* 45: 622–629.
- McKAY, J. K., C. E. CHRISTIAN, S. HARRISON, AND K. J. RICE. 2005. “How local is local?”—A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 13: 432–440.
- MERILA, J., AND P. CRNOKRAK. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* 14: 892–903.
- MONTALVO, A. M., AND N. C. ELLSTRAND. 2000. Transplantation in the subshrub *Lotus scoparius*: I. Test of the home site advantage hypothesis. *Conservation Biology* 14: 1034–1045.
- MONTALVO, A. M., AND N. C. ELLSTRAND. 2001. Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaceae). *American Journal of Botany* 88: 258–269.
- MONTALVO, A. M., S. L. WILLIAMS, K. J. RICE, S. L. BUCHMANN, C. CORY, S. N. HANDEL, G. P. NABHAN, ET AL. 1997. Restoration biology: A population biology perspective. *Restoration Ecology* 5: 277–290.
- OOSTERMEIJER, J. G. B., R. G. M. ALTENBURG, AND H. C. M. DENNIJS. 1995. Effects of outcrossing distance and selfing on fitness components in the rare *Gentiana pneumonanthe* (Gentianaceae). *Acta Botanica Neerlandica* 44: 257–268.
- PÉLABON, C., M. L. CARLSON, T. F. HANSEN, AND W. S. ARMBRUSTER. 2005. Effects of crossing distance on offspring fitness and developmental stability in *Dalechampia scandens* (Euphorbiaceae). *American Journal of Botany* 92: 842–851.
- PETIT, C., H. FREVILLE, A. MIGNOT, B. COLAS, M. RIBA, E. IMBERT, S. HURTREZ-BOUSSES, ET AL. 2001. Gene flow and local adaptation in two endemic plant species. *Biological Conservation* 100: 21–34.
- RAABOVA, J., Z. MUENZBERGOVA, AND M. FISCHER. 2007. Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus*. *Biological Conservation* 139: 348–357.
- ROSS, M. D. 1973. Inheritance of self-incompatibility in *Plantago lanceolata*. *Heredity* 30: 169–176.
- SHERIDAN, P. M., AND D. N. KAROWE. 2000. Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae), in Virginia. *American Journal of Botany* 87: 1628–1633.
- TONSOR, S. J. 1985. Intrapopulational variation in pollen-mediated gene flow in *Plantago lanceolata* L. *Evolution* 39: 775–782.
- VAN TIENDEREN, P. H. 1992. Variation in a population of *Plantago lanceolata* along a topographical gradient. *Oikos* 64: 560–572.
- WASER, N. M., AND M. V. PRICE. 1985. Reciprocal transplant experiments with *Delphinium nelsonii* (Ranunculaceae): Evidence for local adaptation. *American Journal of Botany* 72: 1726–1732.
- WASER, N. M., AND M. V. PRICE. 1994. Crossing-distance effects in *Delphinium nelsonii*: Outbreeding and inbreeding depression in progeny fitness. *Evolution* 48: 842–852.
- WILLI, Y., M. VAN KLEUNEN, S. DIETRICH, AND M. FISCHER. 2007. Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. *Proceedings of the Royal Society of London, B, Biological Sciences* 274: 2357–2364.